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# Obese rats are more vulnerable to inflammation, genotoxicity and oxidative stress induced by coal dust inhalation than non-obese rats

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ABSTRACT

Obesity is an important nutritional disorder worldwide. Its association with environmental pollution may trigger an increase in oxidative stress and inflammatory parameters. Coal is a resource used throughout the world as an important fuel source for generating electricity. The ashes released by the coal combustion cause serious problems for human health due to their high toxicity and their capacity to bioaccumulate. The aim of this work was to investigate the effects of coal dust inhalation in the organs of obese and non-obese Wistar rats. Pro-inflammatory cytokines, oxidative stress, oxidative damage, histological analysis, comet assay, and micronuclei were investigated. Both obesity and coal dust inhalation increased the pro-inflammatory cytokines IL-1β and TNF-α and decreased HSP70 levels in serum, however, in obese animals that inhaled coal dust these changes were more pronounced. Liver histological analysis showed severe microvesicular steatosis in obese animals that inhaled coal dust. Lung histologic investigation showed abnormalities in lung structure of animals exposed to coal dust and showed severe lung distensibility in obese animals exposed to coal dust. The comet assay showed DNA damage in animals subjected to coal. In addition, there were modulations in enzymatic activities and damage to protein and lipids. Based on our results, the coal dust inhalation can potentiate the pro-inflammatory profile present in obese rats. We also observed an increase in the protein oxidative damage in obese rats that inhaled coal dust. Taken together, our results suggest that the combination of obesity and coal inhalation increased the risks of the development of diseases related to oxidative stress and inflammation.

## 1. Introduction

Obesity is a worldwide disorder and is associated with cardiovascular disease, diabetes and cognitive disorders (Rummel et al., 2016). Data from the World Health Organization (WHO, 2005) estimate that by 2025 more than 2.3 billion adults will be overweight and over 700 million, obese. Genetic, environmental and behavioral factors are important contributors to the development of this condition. The causes of the current obesity epidemic are associated with sedentary lifestyles, increased consumption of unhealthy foods and reduced physical activity (Awada et al., 2013).

Obesity is characterized by excessive storage of adipose tissue (Fernández-Sánchez et al., 2011). Adipose tissue acts as an endocrine organ generating a chronic low-grade inflammation and releasing a variety of bioactive adipokines, including leptin and adiponectin, which are implicated in the homeostasis of physiological and pathological processes involving reactive species production (Marseglia et al., 2015). Overproduction of reactive species may result in oxidative stress and

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damage to cellular structures, thus leading to the development of pathologies.

Coal is an important source of fuel for the generation of electrical energy around the world, influencing various socio-environmental factors and affecting human health. It is known that during coal burning, large amounts of dust particles and particulate matter is produced and released into the air.

The inhalation of harmful substances such as coal dust present in the air represents a silent risk factor to a healthy human (Gasparotto et al., 2013; Leon-Mejia et al., 2014). The continuous inhalation of coal dust is hazardous and may lead to oxidative stress, oxidative damage, acute pro-inflammatory response and damage to macromolecules such as lipids (lipid peroxidation), proteins, carbohydrates and nucleic acids (DNA) (da Silva, 2016; Leon-Mejia et al., 2018; Schins and Borm, 1999).

Lung is the first organ directly exposed to coal dust. In addition, the liver is a vulnerable target organ since inhaled coal dust may be translocated from the extra-pulmonary circulation crossing the alveolar epithelial layer reaching the bloodstream, and the liver microvasculature allows ready access of these nano-constituents to hepatocytes (Bourdon et al., 2012; Kim et al., 2014; Mani et al., 2007). The association between obesity and coal dust inhalation may induce organ-specific pathologies or even aggravate existing diseases where obesity is present. Our hypothesis is that organs with excessive storage of adipose tissue may be more vulnerable and prone to pathologies development.

The aim of the present study was to investigate whether obesity could be an aggravating factor of coal pollution-mediated damage or not. To achieve this objective, we investigate the oxidative damage and oxidative stress induced by a high-fat diet (HFD), coal dust inhalation (CDI), and the associated treatments (HFD + CDI) on serum, blood, liver, lung tissue using a rat experimental model. We also evaluate omental fat, retroperitoneal fat, visceral fat, brown adipose tissue (BAT), all of which characterize obesity. Histologic analysis and DNA damage assay were performed to explain the effects of coal dust inhalation on obese and non-obese rats.

To induce obesity, animals received an HFD (60% of fat) for six months (Bortolin et al., 2018). Diets containing more than 30% fat can induce obesity (Ghibaudi et al., 2002; Hariri and Thibault, 2010). The obesity model was confirmed by significantly increased body weight and specific fat storing tissues in animals subjected to HFD, in comparison to the animals that received a standard bioterial diet. In addition, the HFD induced insulin resistance was evaluated using glycemic levels. Rats that received HFD had high levels of glycemia.

## 2. Materials and methods

### 2.1. Chemicals

The chemicals used in the study were as follows: Glycine, hydrogen peroxide ($H_2O_2$), catalase (CAT, EC 1.11.1.6), superoxide dismutase (SOD, EC 1.15.1.1), epinephrine, nicotinamide adenine dinucleotide phosphate (NADPH), 1-Chloro-2,4-dinitrobenzene (CDNB), trichloroacetic acid (TCA), 2,4-dinitrophenylhydrazine (DNPH), 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB), thiobarbituric acid (TBA), Sodium chloride (NaCl), Ethylenediaminetetraacetic acid (EDTA), dimethyl sulfoxide (DMSO), glycerol. Polyclonal and monoclonal antibodies from Abcam® (Cambridge, UK), Tumor necrosis factor-α (TNF-α)-(ab6671), Interleukin-1β (1IL-1β)-(ab9722) HSP70 (4872). Anti-Rabbit IgG, peroxidase conjugated (#AP132P) and anti-Mouse IgG, peroxidase-conjugated and H + L from (#AP124P) Merck (Massachusetts, EUA).

MilliQ-purified H2O was used for preparing solutions. ELISA microplates were from Greiner Bio-One (Monroe, USA) and ELISA TMB spectrophotometric detection kit was from BD Biosciences (San Diego, USA).

### 2.2. Ethics statement

All animal experimental procedures were performed in accordance with the Committee of Ethics for the Use of Animals (CEUA) of the Federal University of Rio Grande do Sul (UFRGS) and laws of the use and handling animals in research according to the Federal Law number 11794 of 8 October 2008/resolution 879 of February 15, 2008. CEUA protocol number: 25739.

### 2.3. Animals

Sixty-day-old male Wistar rats of 250–300 g weight were obtained from our breeding colony. They were maintained in a cycle of 12 h light/dark at a constant temperature of 22 °C ± 2. They were housed in polypropylene boxes (41 × 34 × 16 cm), four (4) animals per box and were cleaned and changed three times a week. Animals had free access (*ad libitum)* to water.

### 2.4. Coal dust

The coal sample was purchased from the largest thermoelectric plant in Latin America, Tractebel Suez, located in Santa Catarina, Brazil, and its chemical composition itemized (Silva et al., 2010).

### 2.5. Experimental model

Firstly, animals were divided into two groups ($n$ = 16 per group). Diets were administered for five months as follows:

*Group 1 (control):* had free access to commercial food (Chow Nuvilab CR-1 type; Curitiba, PR, Brazil). Chow nutritional composition includes total protein (22%), vegetal fiber (8%), minerals (10%), calcium (1.4%), and phosphorous (0.8%). Enrichment by kilograms: vitamin A (12,000 IU), vitamin D3 (1800 IU), vitamin E (30 IU), vitamin K3 (3 mg), vitamin B1 (5 mg), vitamin B2 (6 mg),vitamin B6 (7 mg), vitamin B12 (20 μg), niacin (60 mg), folic acid (1 mg), biotin (0.05 mg), choline (600 mg), iron (50 mg), copper (10 mg), zinc (60 mg), manganese (60 mg), cobalt (1.5 mg), iodine (2 mg), selenium (0.05 mg), lysine (100 mg), and methionine (300 mg).

*Group 2:* Received a high-fat diet (HFD) as described by de Assis et al. (2009). HFD contained 60% fat, composed of 59% lard and 1% soybean oil, 20% protein, 15% carbohydrates and 5% mineral salts.

After 5 months of diet administration, the animals were divided into four groups:

I) Control unexposed to CDI and non-obese (control $n$ = 8);
II) Coal dust inhalation (CDI, $n$ = 8);
III) HFD unexposed to CDI (HFD, $n$ = 8);
IV) HFD + CDI ($n$ = 8).

Diets were maintained during exposure to the coal dust (1 month), totaling 6 months of treatment. The food intake was monitored three times a week and body weight measured once a week.

### 2.6. Rats exposed to coal dust inhalation

Animals were exposed to mineral coal (10 mg/m$^3$) 3 h per day totaling 30 days (Mani et al., 2007). Glass inhalation chambers were used for the whole body with 21 L of capacity and an internal diameter of 30 cm. The coal aerosol was generated from a generator with a 15 L flow rate/min (Supplementary Fig. 1). The concentrations were continuously recorded using a 25 mm filter (Intox Products, USA).

### 2.7. Tissue samples

24 h after the final inhalation, the animals were decapitated using a guillotine and blood, liver, omental fat, retroperitoneal fat, visceral fat,